

A Standardized and Well-Characterized MSC Source for Translational Researchers

RoosterBio

Lye Theng Lock, PhD¹; Iain Farrance, PhD¹; Priya Baraniak, PhD¹; Jian-Jiang Hao, PhD²; Jon Rowley, PhD¹

1. RoosterBio, Inc. 4539 Metropolitan Court, Frederick, MD 21704; 2. Poochon Scientific, 4539 Metropolitan Court, Frederick, MD 21704

ABSTRACT

Bone marrow-derived Mesenchymal Stem Cells, or MSCs, have been recognized as potential patient-specific drugstores, and will soon be the key raw material for future therapeutics, engineered tissues, and medical devices. There are currently over 400 global clinical trials investigating MSCs as therapies across a host of diseases, and results have been encouraging, with MSCs deemed safe for transplantation. However, the resulting increased demand for research- and clinical-grade MSCs is not met by current commercial suppliers, and many researchers must isolate and manufacture their cells in-house, resulting in MSCs that are fraught with variability and processes that are non-scalable. RoosterBio Inc. is addressing these challenges by offering well-characterized MSCs that are manufactured with scalable, GMP-compatible bioprocesses. RoosterBio has streamlined and simplified the MSC culture process allowing for more rapid cell production at much lower overall costs. RoosterBio MSCs have superb growth characteristics compared to MSCs grown with traditional media and culture systems, and achieve a 200- vs. 3-fold expansion within 10 days of culture. RoosterBio MSC lots are well-characterized for their cell surface marker expression (CD73, 90, 105, and 166), angiogenic cytokine secretion (VEGF, IL8, TIMP1, TIMP2, FGF, HGF), immunomodulatory function (inducible IDO expression), as well as their differentiation potential into both bone and fat lineages. In addition, RoosterBio's highly controlled and streamlined culture processes result in a product with much less donor-to-donor variability in growth kinetics, thereby providing more standardized and affordable off-the-shelf MSCs for product developers and translational researchers.

MSC EXPANSION KINETICS

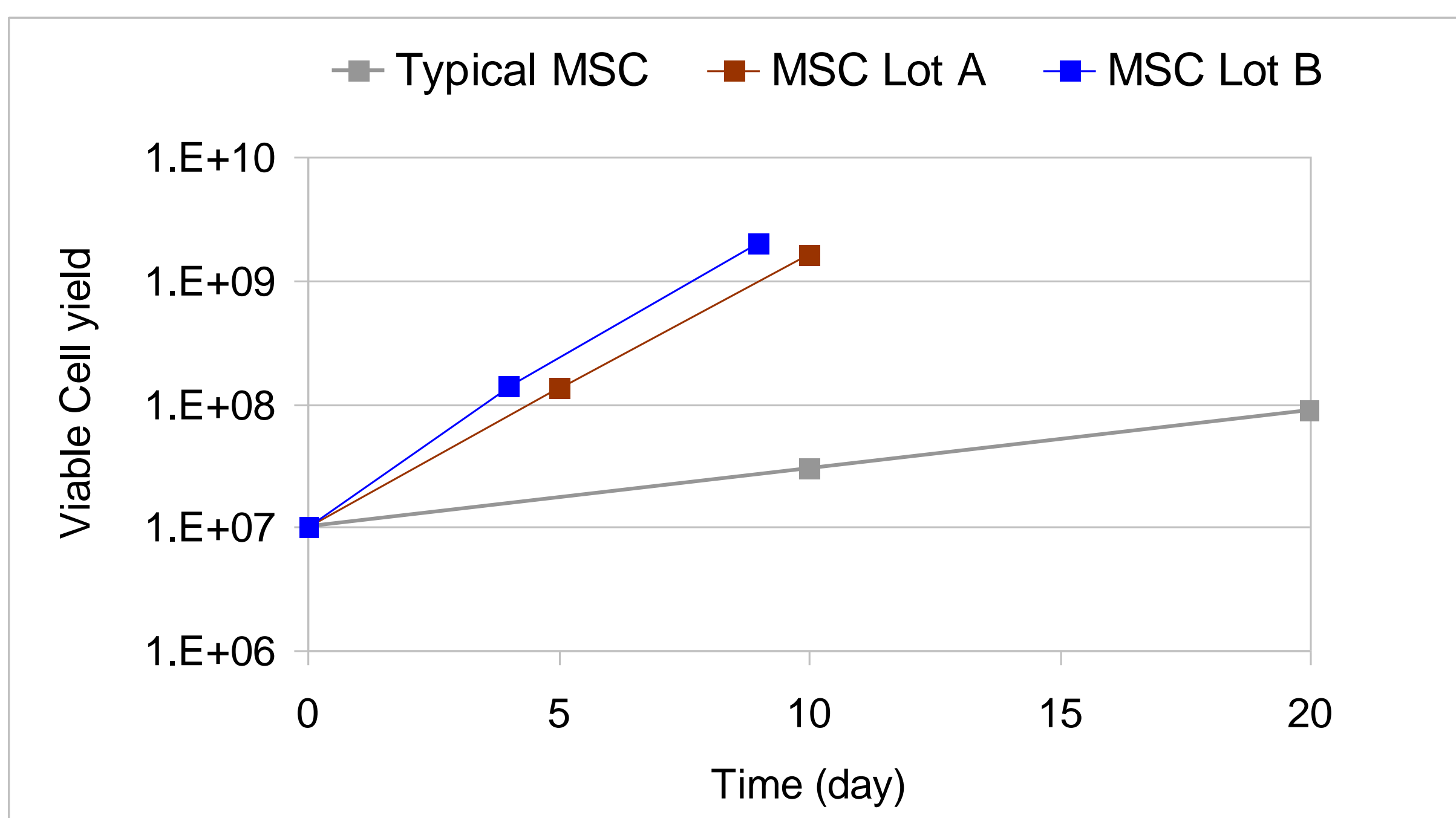


Fig. 1: Expansion profile of RoosterBio MSCs (Lots A and B) compared to typical MSC culture in 10% FBS + DMEM. RoosterBio MSCs show accelerated cell expansion (10-fold increase in cell yield within 4-5 days vs. 20 days for MSCs in conventional culture media).

MSC MULTILINEAGE POTENTIAL

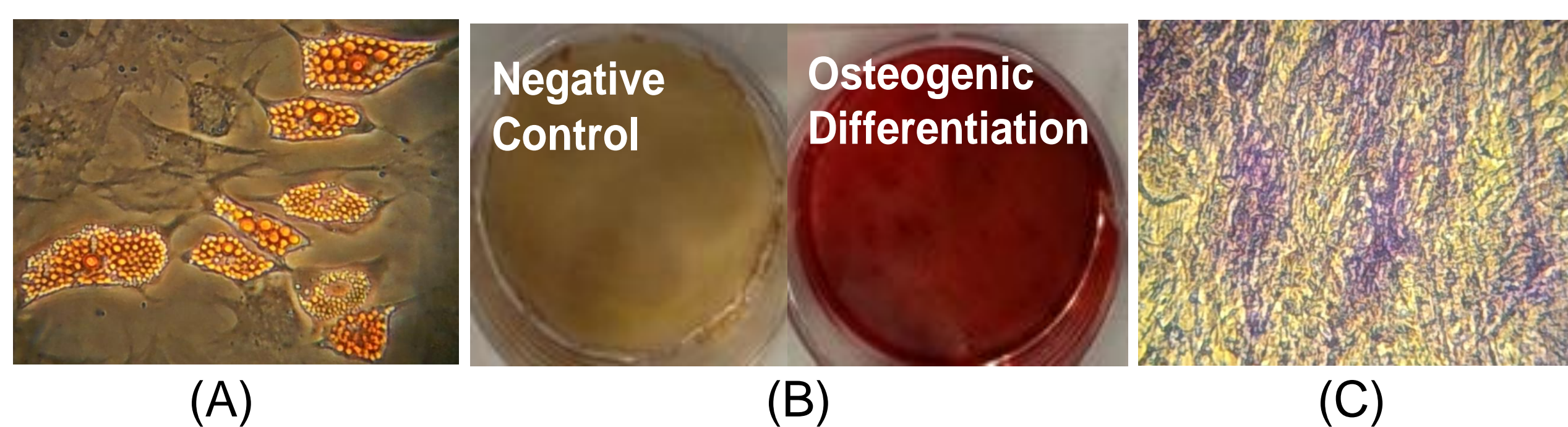


Fig. 2: RoosterBio MSCs differentiate into (A) adipocytes (evidenced by oil droplet staining of lipid vacuoles) and osteocytes (evidenced by (B) Alizarin red and (C) alkaline phosphatase staining).

MSC SURFACE MARKER PROFILE

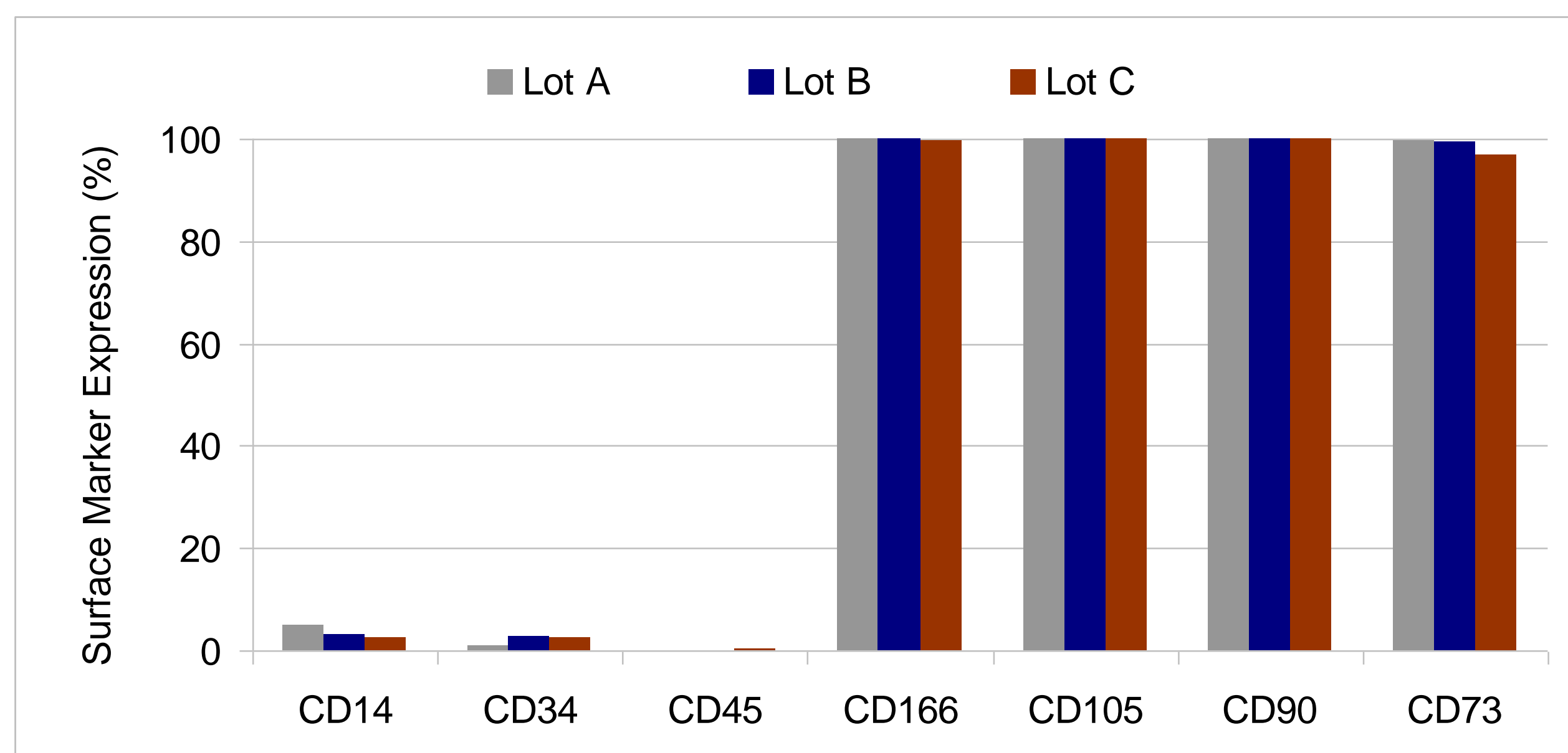


Fig. 3: MSCs were >95% positive for stromal cell surface marker expression (CD73, 90, 105, and 166) and did not express (<5% positive) non-stromal surface markers (CD14, CD34 and CD45).

MSC CYTOKINE SECRETION

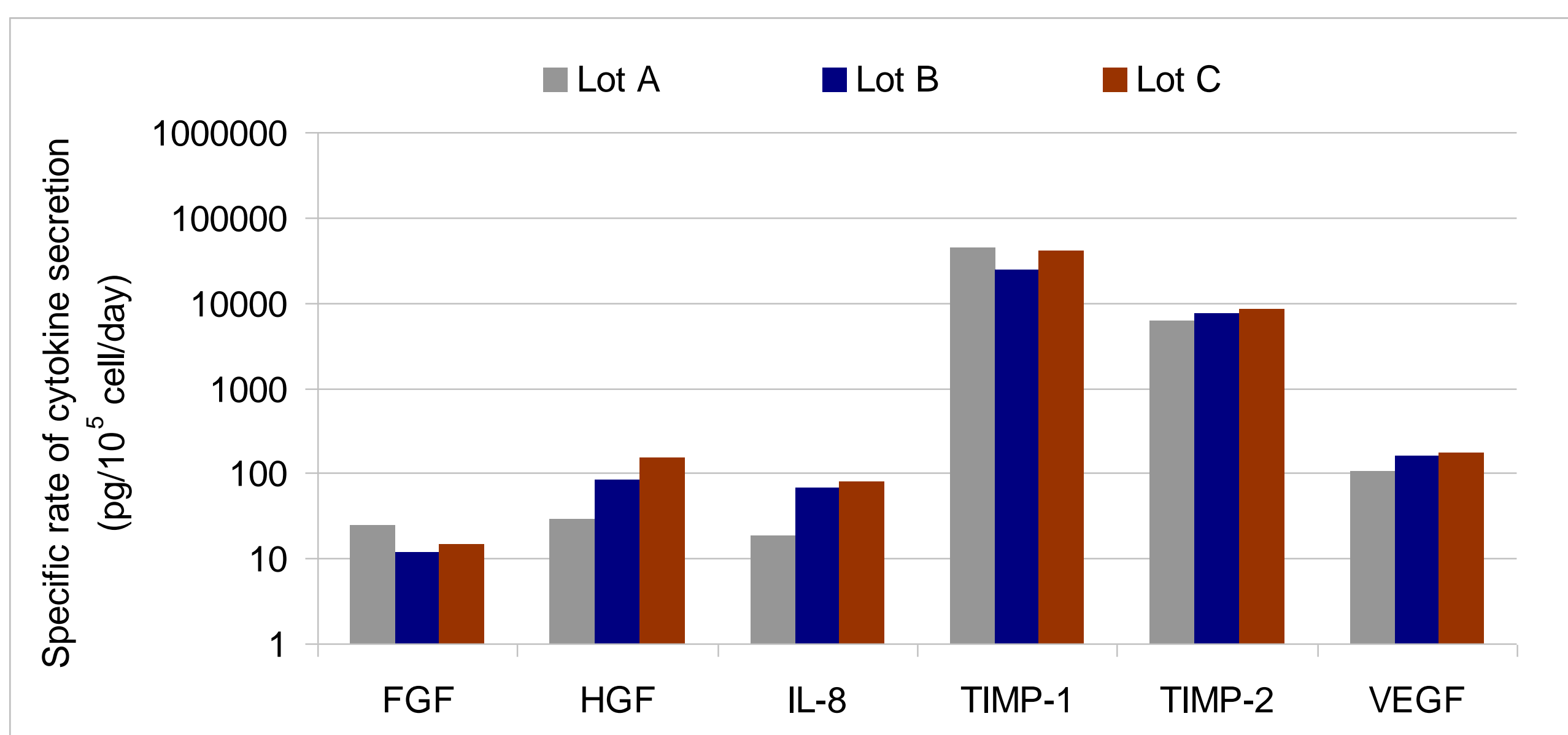


Fig. 4: Cells secreted clinically-relevant angiogenic cytokines (FGF, HGF, IL8, TIMP1, TIMP2, and VEGF) 5 days post-expansion in RoosterBio media.

IMMUNOMODULATORY RESPONSE

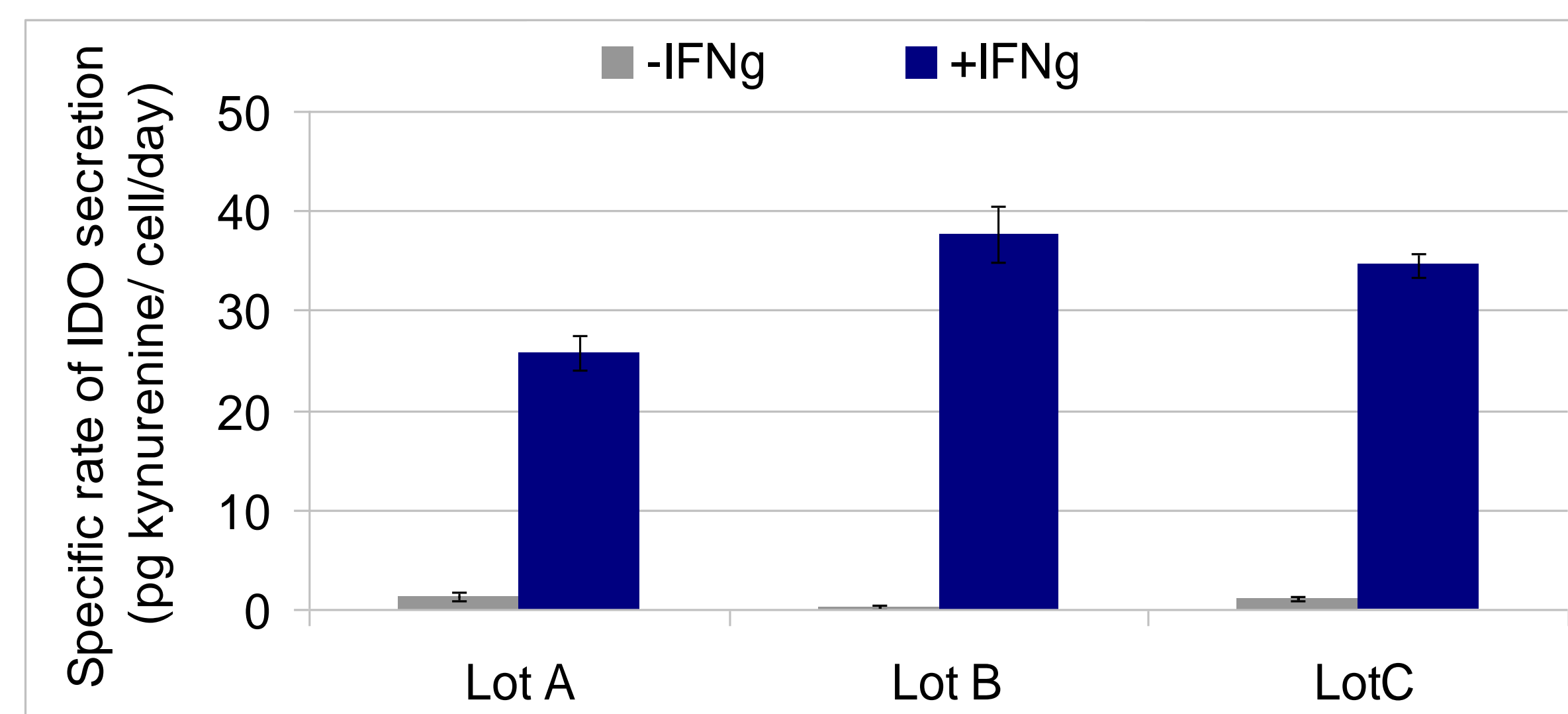


Fig. 5: MSCs stimulated with interferon-gamma (+IFNg) upregulated IDO secretion compared to non-stimulated (-IFNg) cells. This is a clinically-relevant potency assay for MSC immunomodulatory function.

MSC WHOLE PROTEOME ANALYSES

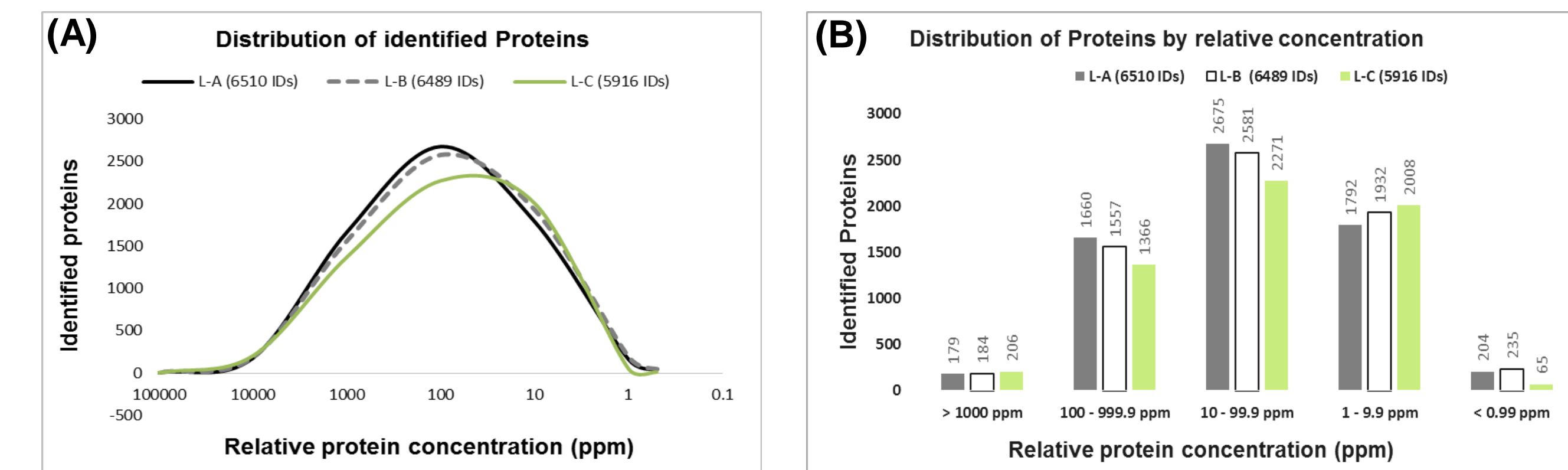


Fig. 6: The distribution of identified proteins in three distinct MSC lots was plotted according to their relative concentration (ppm). The X-Axis is log scale of concentration (ppm), and the Y-Axis is the corresponding number of identified proteins. (A) Plot showing the normal distribution of 6000+ identified proteins for three lots of MSCs; (B) Column plot demonstrating the distribution of identified proteins by their range of relative protein concentration (ppm).

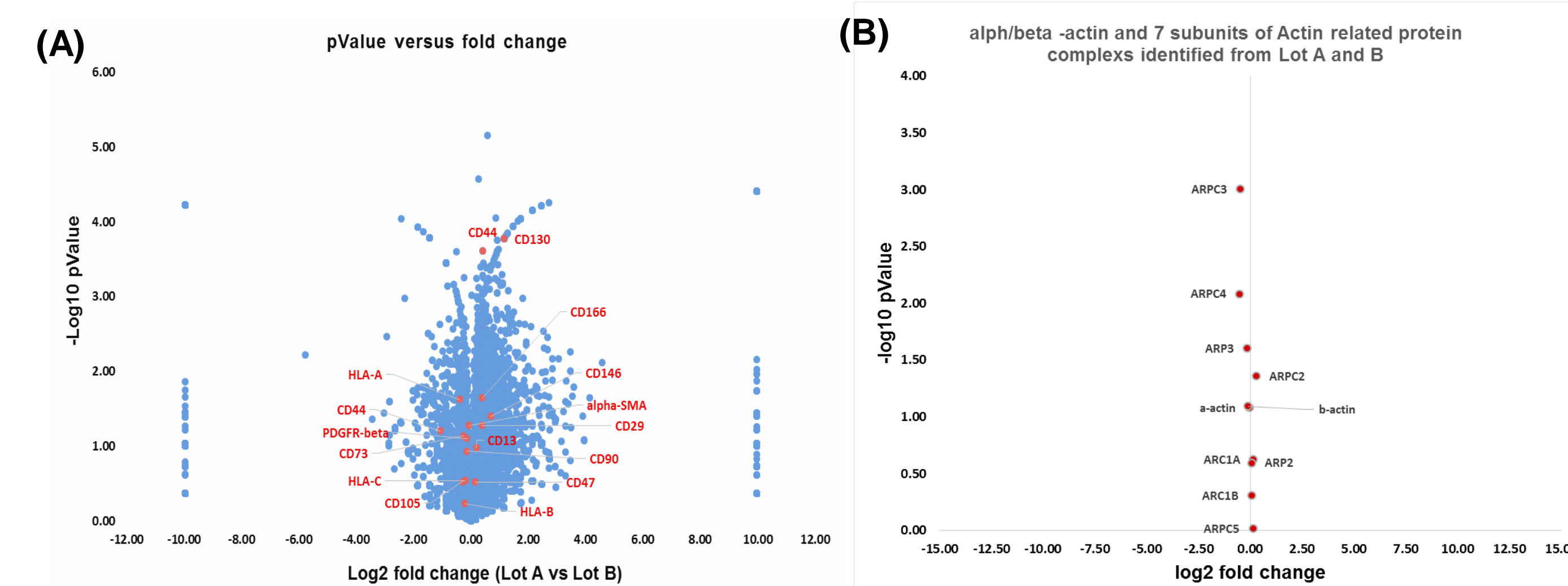


Fig. 7: (A) Volcano plot of proteomic profiles between two lots of MSCs demonstrates great similarity between the donor cell lots. The X-axis represents fold-changes of lot A versus lot B (log₂ of fold change), and the Y-axis represents the statistical significance p-value (-log₁₀ of p-value). Red dots represent selected MSC markers identified in both lots with no change. (B) Similar levels of cytoskeleton regulator Arp2/3 complex subunits and alpha- and beta-actin (i.e. housekeeping proteins) are also seen between MSC lots. Taken together, these data demonstrate little lot-to-lot variability in RoosterBio MSCs.

CURRENT PRODUCT

Donor Information		MSC Product Information	
Age	Gender	Size/vial	PDLs
43	Male	10M	8
33	Male	10M	8
20	Female	10M	8
29	Female	10M	9
25	Male	10M	8
29	Female	25M	16
29	Female	100M	16

RoosterBio Media		MSC Expansion in 4-5 days	
High Performance MSC Media Kit	500ml	>10-fold	